NSLS-II User Workshop

a user point of view

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Macromolecular crystallography

Structure ⇔ function

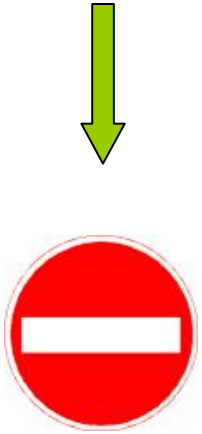
your protein
(substate A)

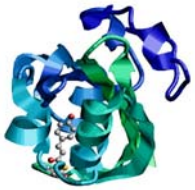
your protein
(substate B)

your protein
(substate C)

Enzyme vs photoreceptor

Enzyme

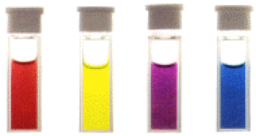




Photoreceptor

I_1

I_2



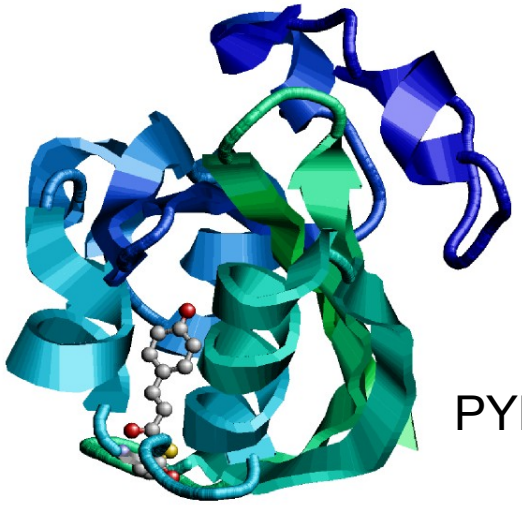
Photoreceptors

- six families : rhodopsins, phytochromes, xanthopsins, cryptochromes, phototropins and BLUF-proteins

- membrane/soluble, single domain/multidomains proteins

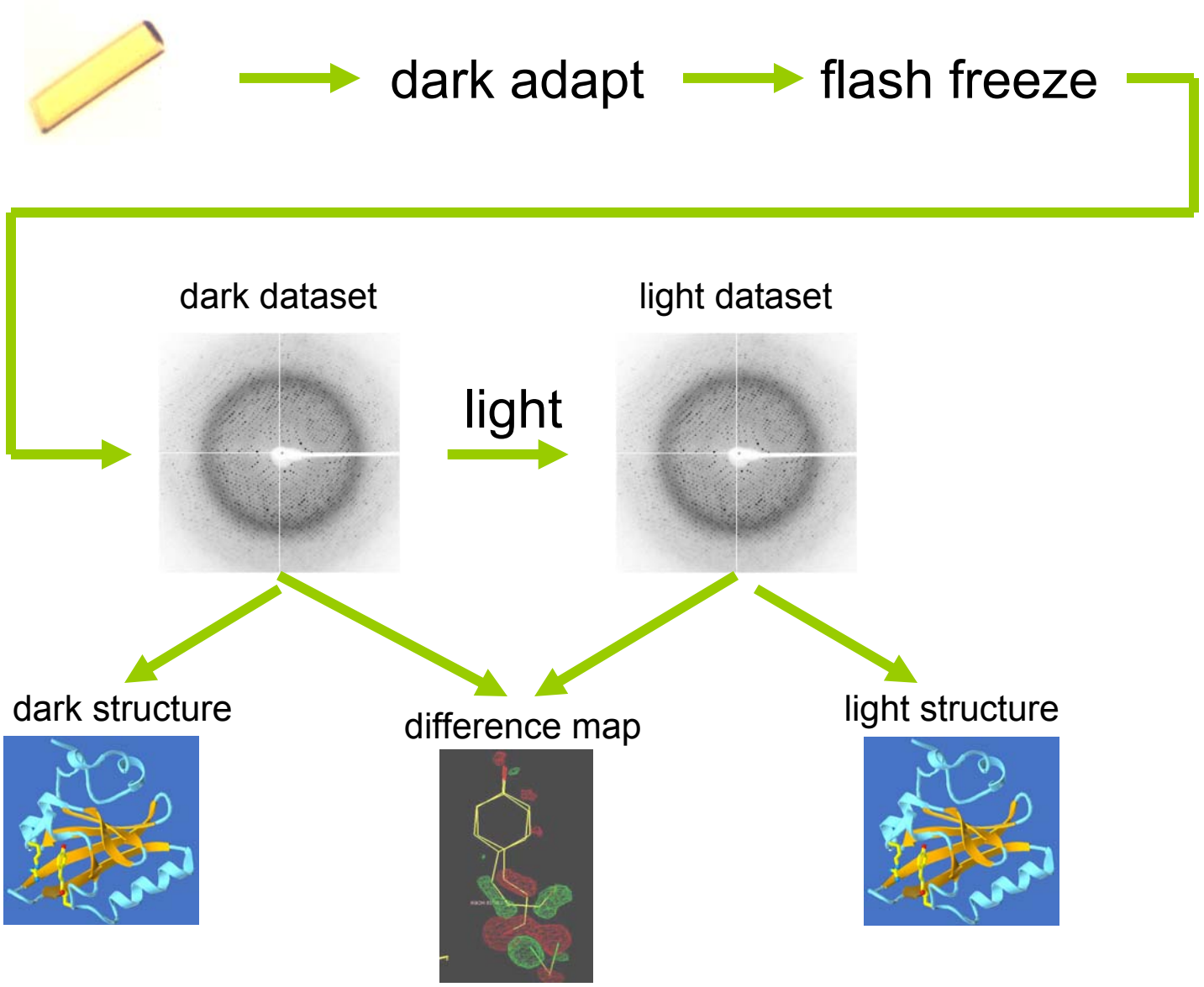
- modulate gene expression, enzyme activity and/or motility

Structure ⟷ Function

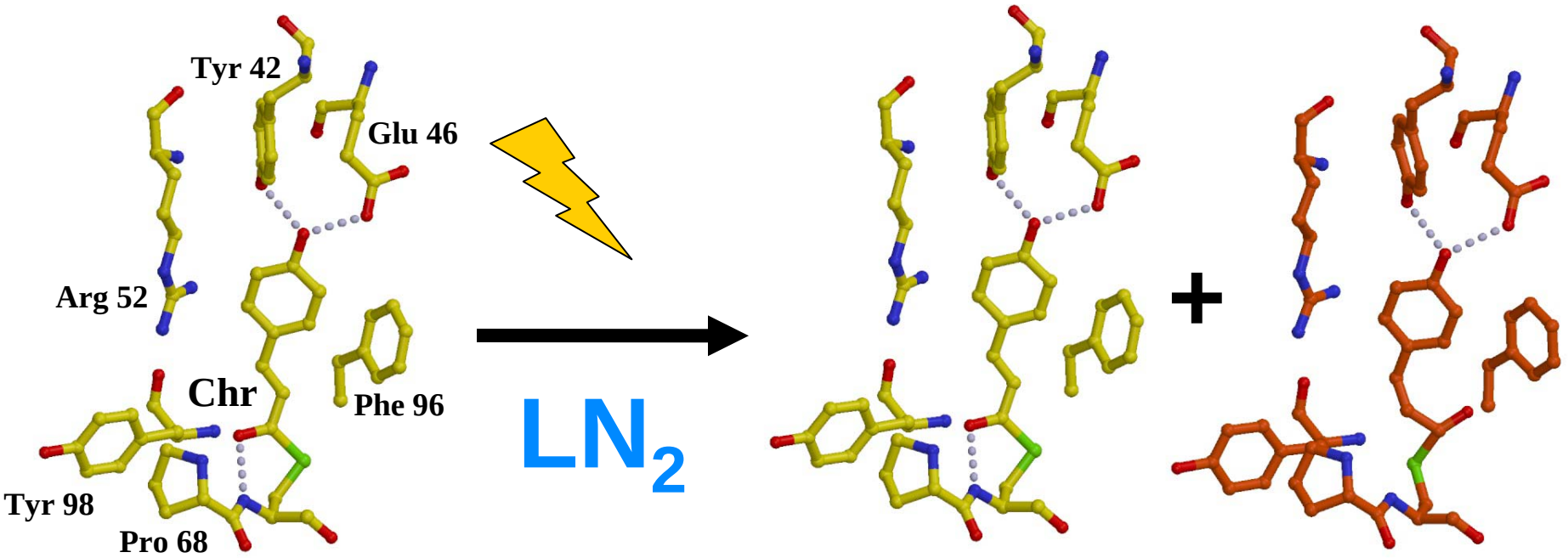


PYP

Typical experiment



Activation results



100 %

Ground state

~70 %

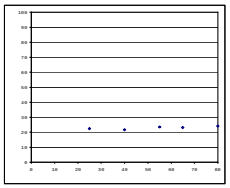
Ground state

~30 %

I_1 intermediate

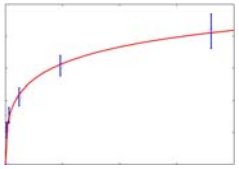
Why is photo-conversion limited?

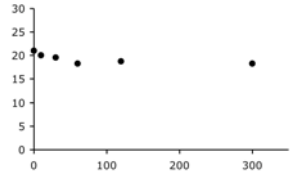
- Not enough light ?



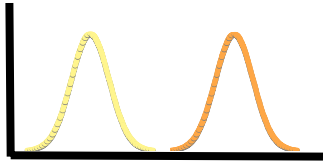
- High optical density of crystal ?

- Dynamic equilibrium ?



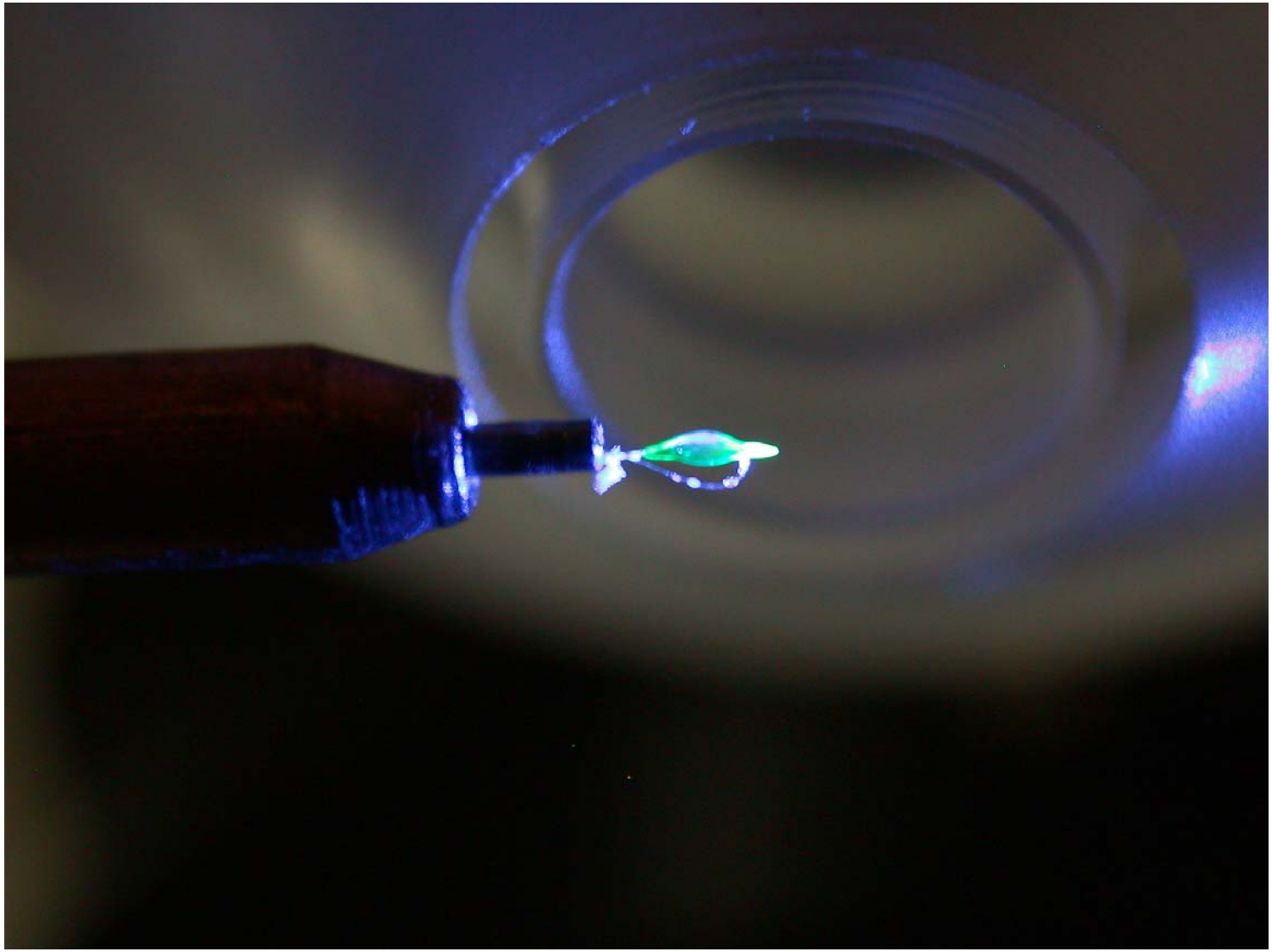


- Photochemical equilibrium ?



- Conformational heterogeneity ?

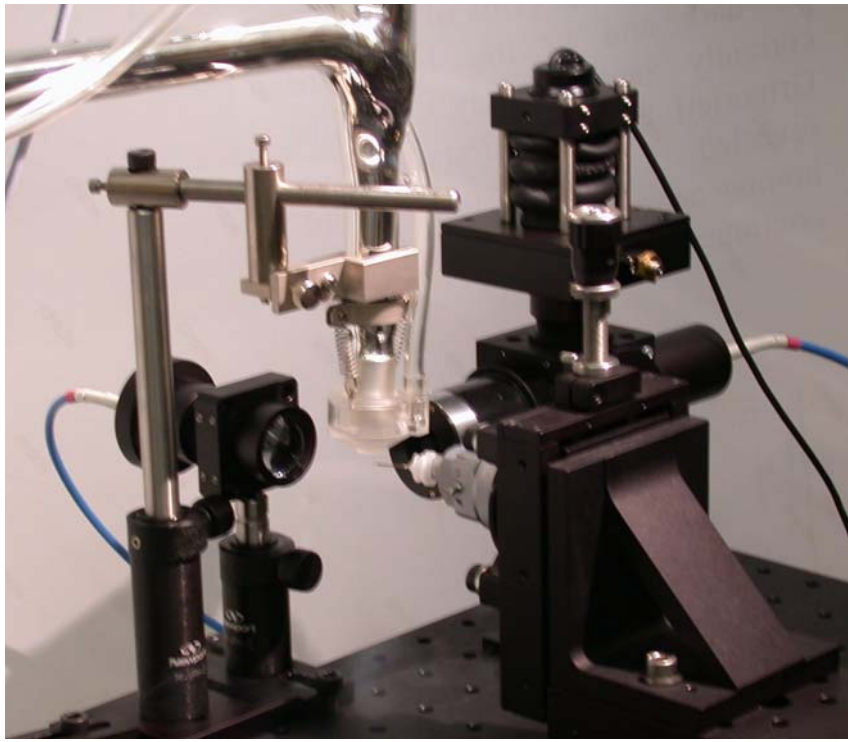
Crystal glowing

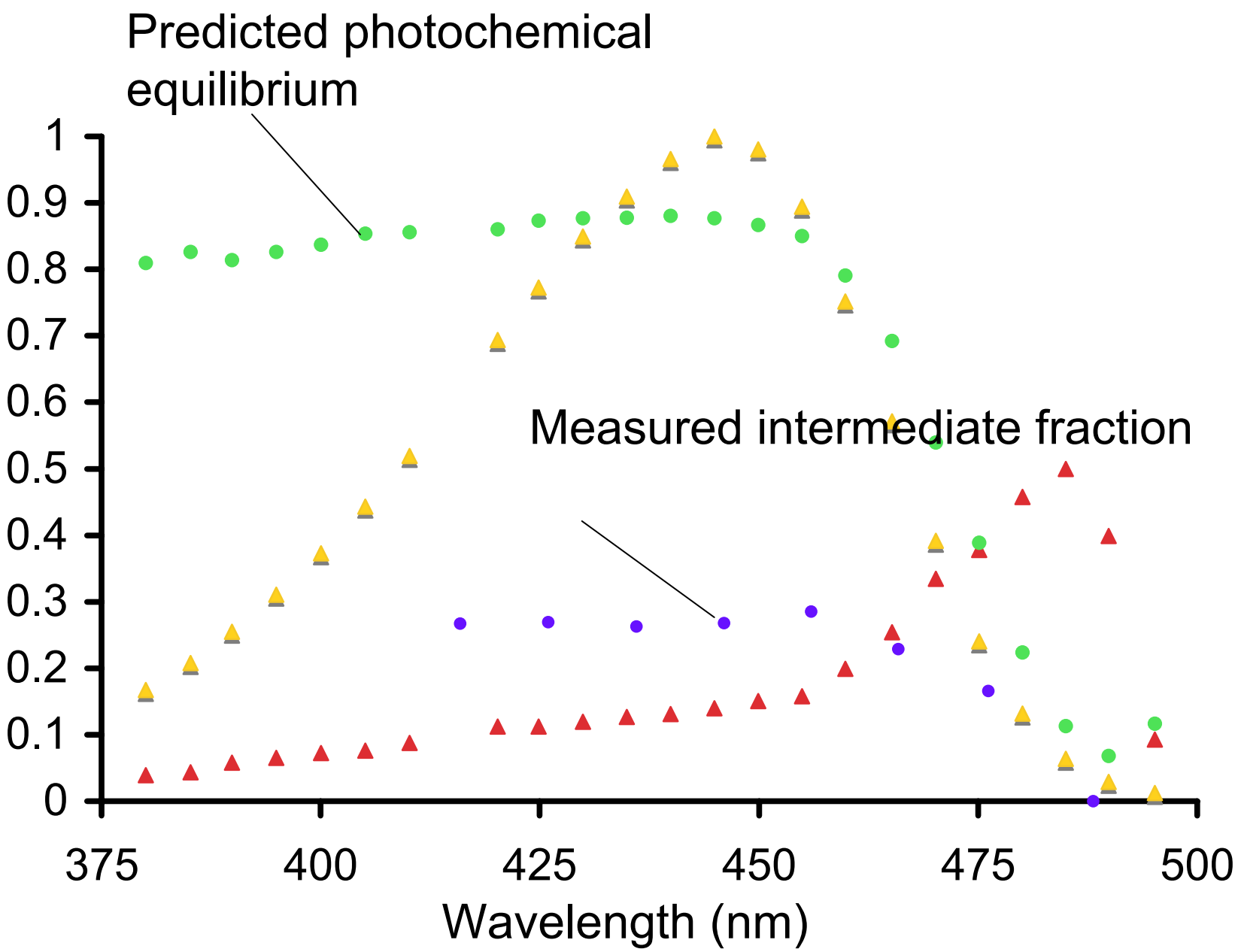


Fluorescence scan

Setup: Single-crystal fluorescence spectrometer

- PYP crystal frozen in the dark
- excitation light
- 100K
- fluorescence detector

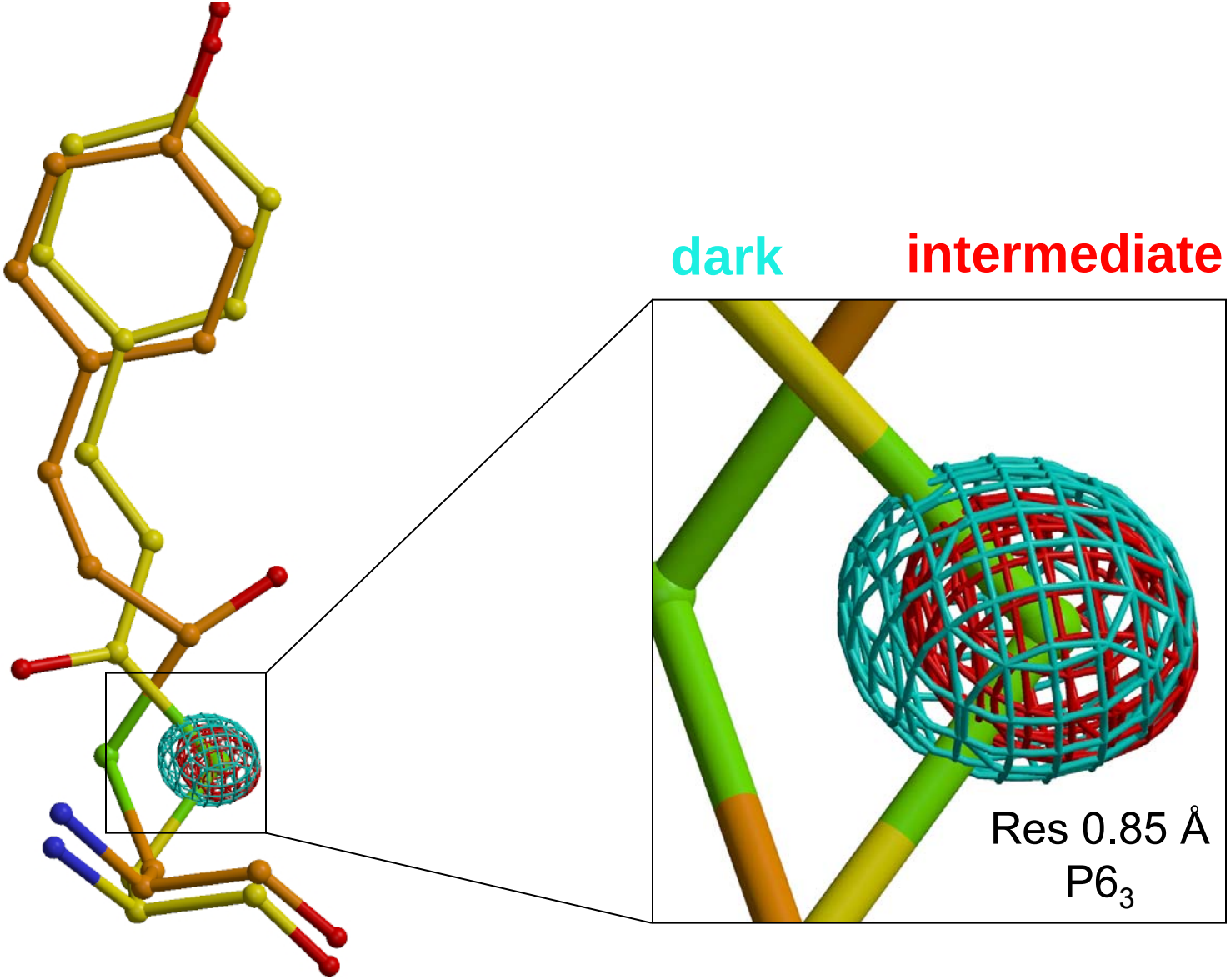



Predicted photochemical equilibrium
Measured intermediate fraction
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
375
400
425
450
475
500
Wavelength (nm)

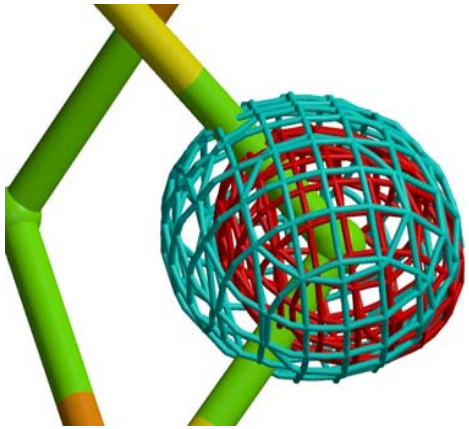
Why is photo-conversion limited?

- Not enough light ✗
- High optical density of crystal ✗
- Dynamic equilibrium ✗
- Photochemical equilibrium ✗
- Conformational heterogeneity 

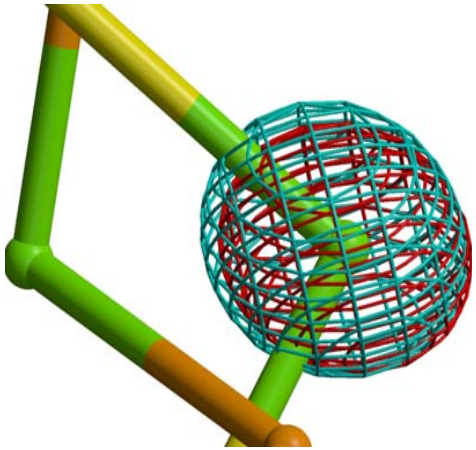
Conformational heterogeneity



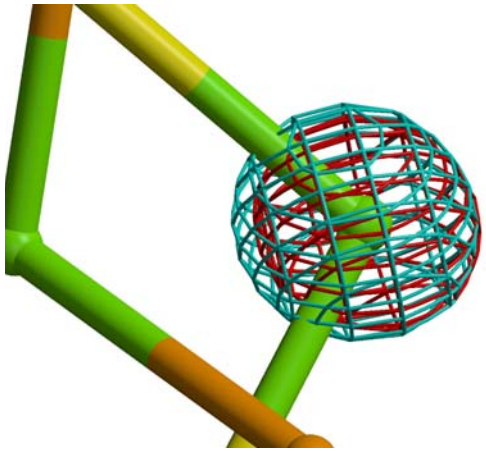
S atom of the chromophore



Res 0.85 Å
$P6_3$

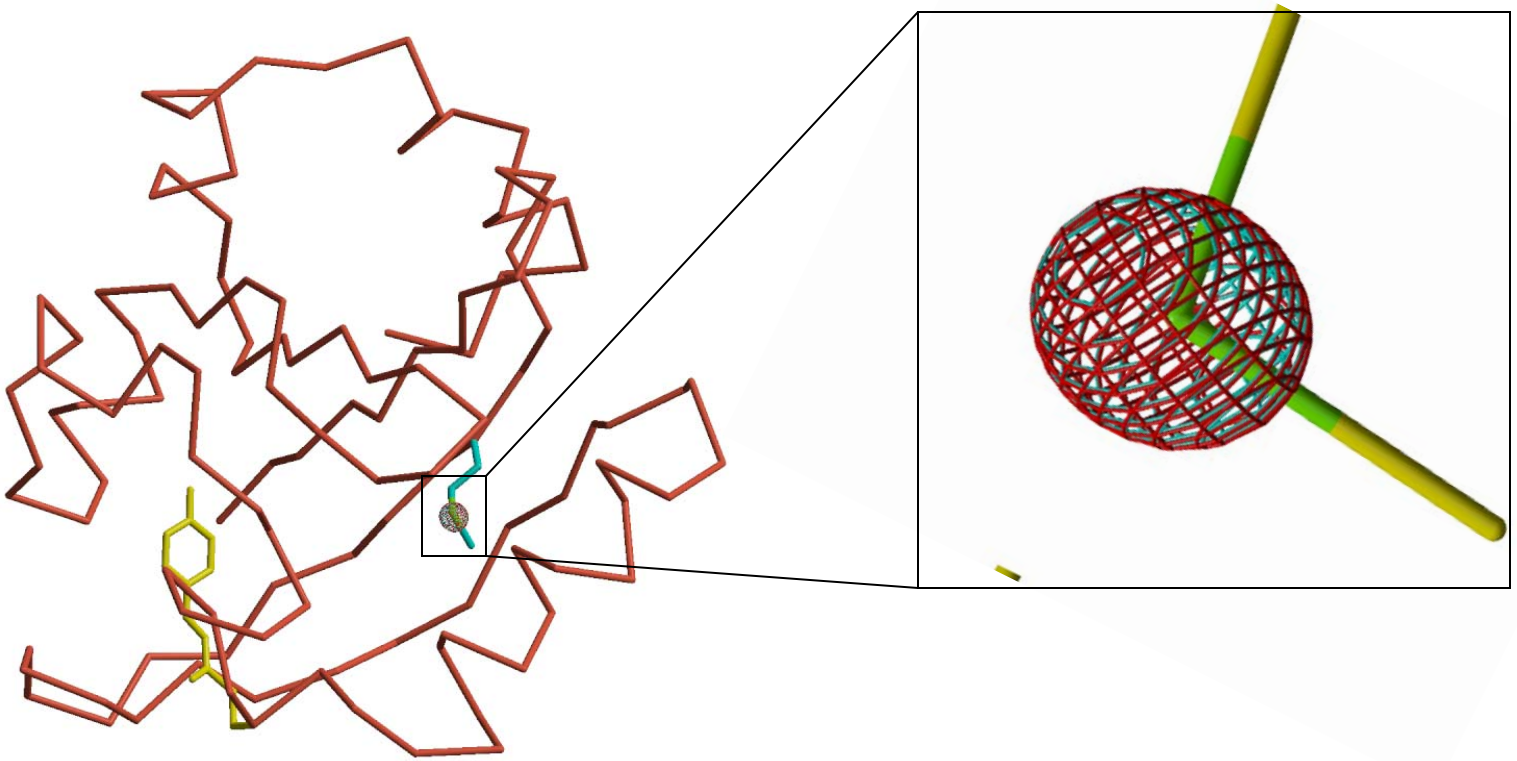


Res 1.15 Å
$P6_5$

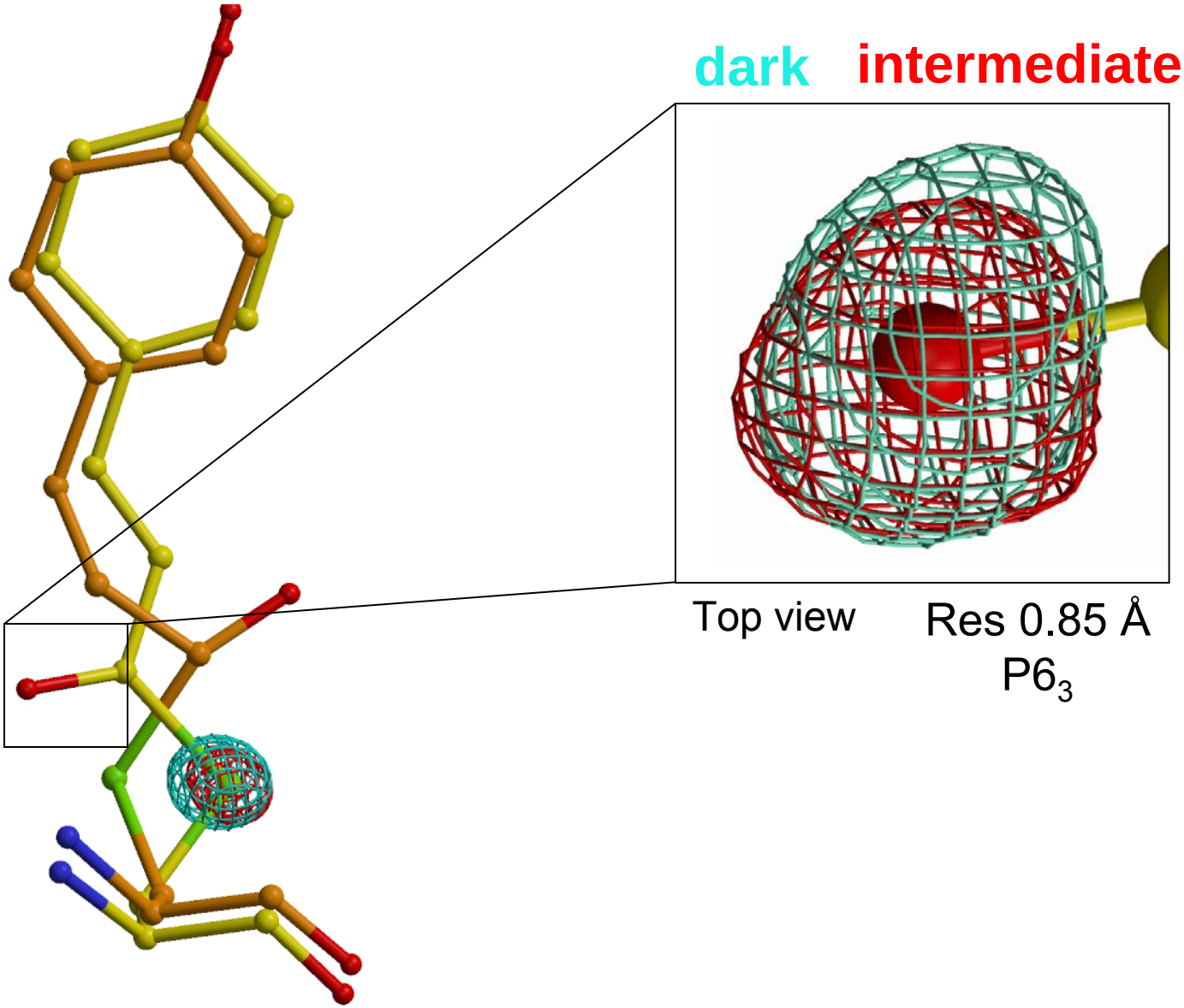


Res 1.2 Å
$P6_5$

Met 109 as a control

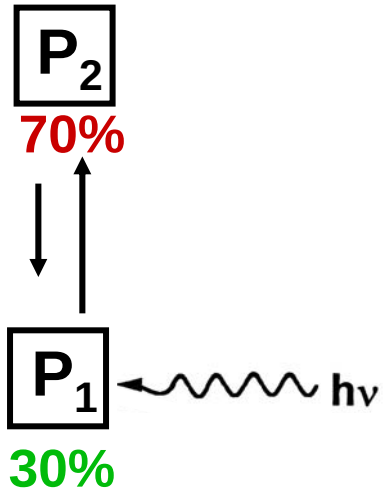


O1 atom in chromophore



Conclusion

- Subatomic conformational change ↔ drastic activity change

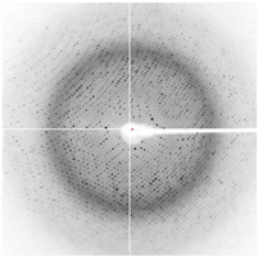


- Crystallographic model corresponds to the inactive form of PYP

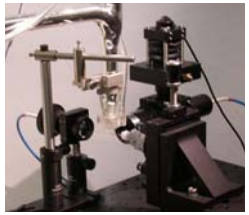
Perspectives

- What I would like to see on a beamline
  - Robot for mounting crystals (limiting time is crystal screening: open door, move detector, mount crystal, close door, shoot crystal)

  - Higher flux and larger detectors
    - Ultra-high resolution
    - Mainly for large complexes



  - Spectroscopic equipment
    - Tunable light source, optic fibers and lenses, laser…
    - Fluorescence detection



Perspectives

– Microfocus beamline
  - Different experiments on the same crystal

– Cryotemperature cooling control
  - Characterize transition states
  - Better control on annealing

– Better dorms
  - (please!!!)

Perspectives

- What I still would like to see on a beamline
  - Remote data collection

  - Mail-in crystals
    - Live interaction with collaborator

  - Fast access
  - Workshop, training
    - Online courses

Acknoledgments

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